APPLIED MICROBIOLOGY, Dec. 1974, p. 1066-1068

Vol. 28, No. 6
Printed in U.S.A.

Growth and Reproduction of Microorganisms Under Extremely Alkaline Conditions

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Received for publication 17 July 1974

An aerobic and an anaerobic strain of bacteria were isolated from two extremely alkaline springs. Growth and reproduction of both microorganisms were demonstrated above pH 11.0.

There are many estimates of the hydrogen ion concentrations below and above which microorganisms cease to grow and reproduce. The highest concentration usually given is 0.1 to 1.0 M (pH 1 to 0) reported for a few fungi and bacteria; the lowest is 10^{-10} to 10^{-11} M (pH 10 to 11), reported for algae, fungi, and various bacterial species (1, 5-8). These estimates are generally based on reports of microorganisms that occur in natural waters of unusually low or high pH. To determine whether organisms actually grow and reproduce at the environmental pH, either in situ or carefully controlled laboratory experiments are needed. Such experiments are lacking, particularly for highly alkaline conditions.

We have examined organisms from two highly alkaline, low-discharge springs to better define the upper pH limit of microbial life. The first spring is located in Stanislaus County, Calif. (Southeast section 15, T. 6S, R. 5E.) in a region characterized by oak-grassland vegetation. The spring comprises a collection of small seeps occurring along a geologic fault. A U.S. Geological Survey mineral analysis of the spring water, made in 1966, showed that the major inorganic constituents were Ca^{2+} (48 mg/liter), Na^+ (40 mg/liter), Cl^- (32 mg/liter), and OH^- (52 mg/liter); the pH was 11.78 (2). At the time of our sampling (1971-72), the pH varied among the small, shallow pools from 11.5 to 11.8. We determined the pH, in situ, with a portable Corning pH meter, model 610A.

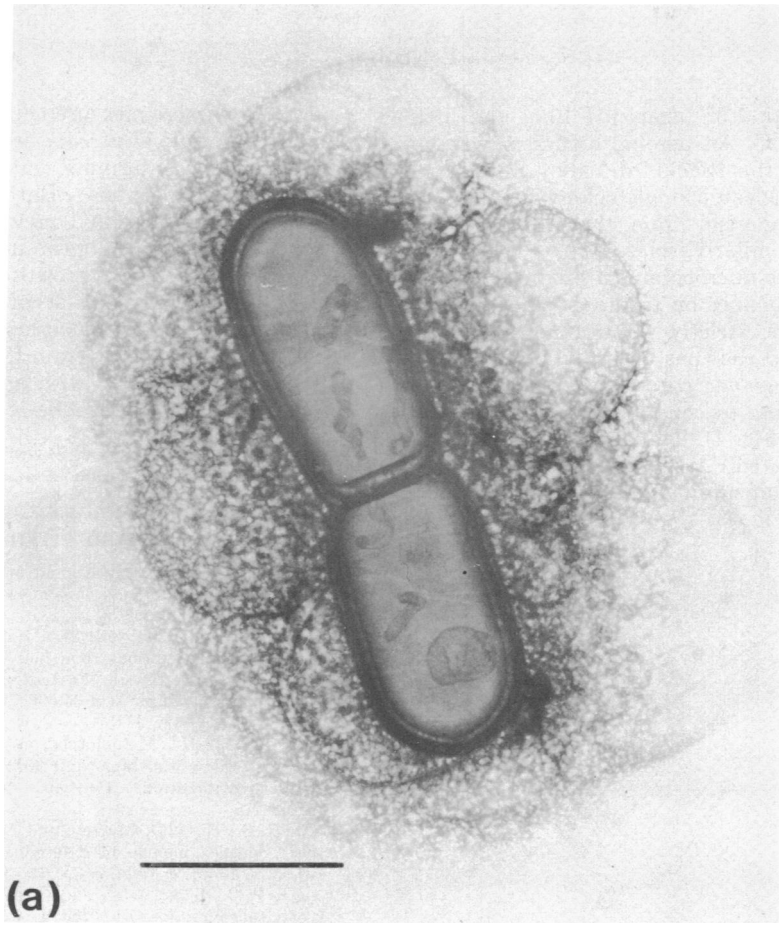
The second spring, Aqua de Ney, is located in Siskiyou County in northern Calif. (Southeast section 32, T. 40N., R. 4W.). The mineral content differs markedly from the Blackbird Valley Spring; the major inorganic constituents are Na^+ (11,300 mg/liter), K^+ (220 mg/liter), Cl^- (7,500 mg/liter), CO^{2-} (5,450 mg/liter), and SiO_2 (4,000 mg/liter) (3). This spring is characterized not only by its high pH but also by its odor, the latter due to significant quantities of H_2S (430 mg/liter), and NH_3 (169 mg/liter) (3). The pH of this spring, recorded over a period of years by the U.S. Geological Survey, was 10.9 (3). Our in situ measurements of the main spring and a number of small seeps ranged from pH 10.8 to 12.1.

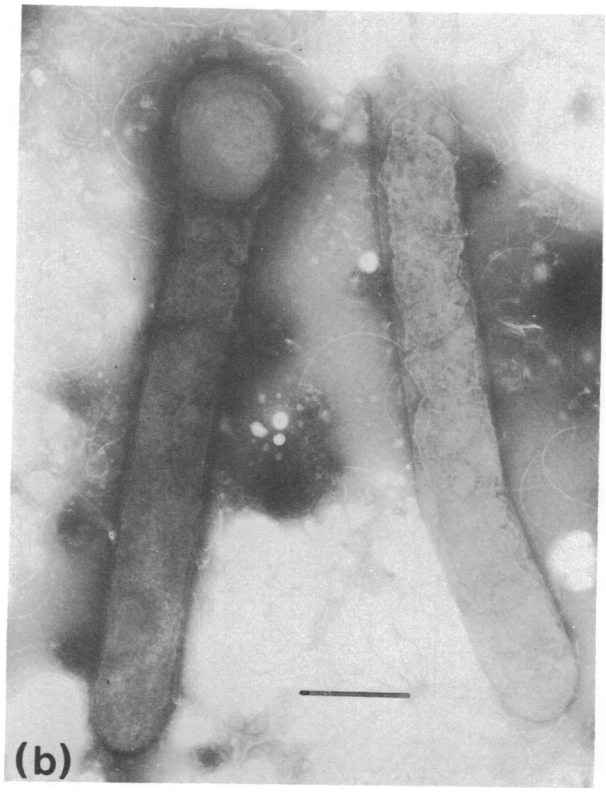
Water and sediment samples were collected from both the Blackbird Valley and Aqua de Ney springs and examined by phase contrast microscopy. Green and blue-green algae, protozoans, and a variety of bacterial forms were observed in the sediment samples of both springs.

The concentration of microorganisms in the water samples from Blackbird Valley was much less than in the sediment samples, whereas no microorganisms were observed in the water samples taken from the main pool at Aqua de Ney. This difference may reflect a greater abundance of nutrients, or lower pH, in the sediments.

Enrichment cultures were started by adding several grams of sediment to 300 ml of 1% tryptic soy broth adjusted to pH 11.0 with 0.1 N NaOH. The suspensions were stirred and incubated at 27 C. The pH was monitored continuously with a Corning pH controller, model 10C, which added 0.1 N NaOH as needed. Both aerobic and anaerobic enrichments were made; mixed populations of bacteria were obtained in both aerobic and anaerobic cultures after several days of incubation. These cultures were streaked onto solid media (1% tryptic soy broth

FIG. 1. *Cell morphology of late-log-phase cells of A-1 (a) and FA-1 (b) grown on 1% tryptic soy broth at pH 10.5. Cells were harvested by centrifugation at 12,100 × g for 10 min at 4 C, washed twice with distilled water, and negative stained with 1% ammonium molybdate, pH 7.2. Solid bars indicate 1 μm.*


(a)


(b)

solidified with 1.5% agar, pH 10.5) and incubated at 30 C. An aerobic organism was obtained from the Blackbird Valley Spring and purified by repeated single-colony isolation. An anaerobic organism from the Aqua de Ney Spring was similarly isolated.

The aerobic microorganism produces orange-pigmented colonies on tryptic soy agar. It is a gram-negative, strictly aerobic, nonsporeforming motile rod that has been tentatively classified as a *Flavobacterium* species (designated A-1), according to common identification procedures (4) (Fig. 1). The anaerobic microorganism, designated FA-1, is a strict anaerobe. It is a gram-variable motile rod that produces spherical spores (Fig. 1).

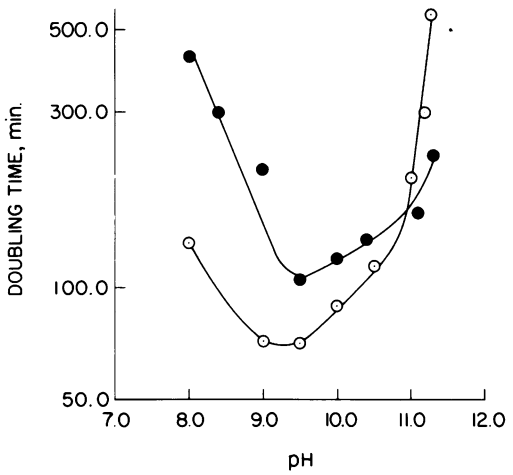


FIG. 2. *Growth rates of A-1 (○) and FA-1 (●) at various alkalinities. A-1 was grown aerobically in 1% tryptic soy broth at 27 C and FA-1 was grown anerobically in 1% tryptic soy broth at 32 C.*

Growth rates were measured either by following changes in optical density at 450 nm or by determining colony-forming units by normal plate-counting methods. Both techniques yielded similar results. A-1 grew over the pH range 8.0 to 11.4 with an optimum between pH 9.0 and 10.0 (Fig. 2). No growth was obtained after a 1-week incubation at pH 7.5 or 11.6, although it survived for much longer periods of time at pH 11.5 to 12.0. Strain FA-1 grew over the pH range 8.0 to 11.3 with an optimum at approximately pH 9.5 (Fig. 2).

We thank Ivan Barnes of the U.S. Geological Survey for information concerning the location and nature of the alkaline springs.

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